Muscle: Fiber & Molecular Mechanics & Structure I

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The Influence of Five Alternative Myosin Converter Domains on Drosophila Muscle Mechanical Properties

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In *Drosophila,* alternatively spliced myosin isoforms containing five different converter sequences (11A-11E) are expressed in a diverse range of muscle types including the superfast indirect flight muscle (IFM), jump muscle and slow embryonic muscles. We measured the converter's influence on IFM fiber mechanical properties such as maximum power output (Pmax), frequency of maximum power output (Fmax), strain for maximum power output (AMP), stretch activated tension (Fsa) and mechanical rate constants 2pib and 2pic. Using the work loop technique to impose muscle strain amplitudes that simulate *in vivo* conditions, we found that transgenically replacing the IFM-11A converter with the embryonic-11D version significantly reduced Pmax by 41%, decreased Fmax by 63%, and increased AMP by 100%. However, no change in Fsa was detected for the IFM fibers expressing the 11D converter. Preliminary results from transgenic substitution of the IFM-11A converter with the embryonic-11E version showed a 50% reduction in Pmax and a 57% reduction in Fmax compared to control muscle fibers. The mechanical rate constant 2pib, determined by sinusoidal analysis, was reduced by 55% by substituting the 11D converter and by 68% by substituting the 11E converter into the IFM myosin isoform. The mechanical rate constant 2pic, increased by more than 50% after substitution of 11D and about 100% with the 11E converter switched into the IFM myosin isoform. These results are physiologically significant as we observed decreased wing beat frequencies of 11% for the 11D, 15% for 11E and decreased flight performance for the 11D fly line at 15°C. We conclude that the five converter versions provide for a wide variation in muscle mechanical properties between muscle fiber types.

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Myosin Kinetics Influence Force Depression in Drosophila Jump Muscle

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Force Depression (FD), a history-dependent phenomenon observed in skeletal muscle, is characterized by a decrease in force production after active shortening compared to an isometric contraction at the corresponding final length. FD measured at steady-state (FDss) increases with larger shortening amplitudes, and decreases with increasing shortening rates. Furthermore, an increase in FDss is associated with a slower rate of force redevelopment following active shortening. Despite these experimental characterizations, the underlying mechanism(s) of FD remain unknown. We have demonstrated that Drosophila's Tergal Depressor of the Trochantor (TDT), or jump muscle, is mechanically similar to mammalian skeletal muscle. More recently, we showed that the TDT exhibits both transient and steady-state aspects of FD, and that both of these aspects, as well as their variations with amplitude and speed of shortening, were similar to mammalian skeletal muscle. Thus, the jump muscle is an excellent model to study FD. When coupled with our ability to genetically manipulate the sarcomeric structure and kinetics through transgene expression, the TDT allows unprecedented insight into the underlying mechanism(s) of FD. This study investigates the effect of slower myosin kinetics on both transient and steady-state aspects of FD in the TDT. A slower embryonic myosin isoform (EMB) has been transgenically expressed in jump muscle. A battery of active shortenings (3 amplitudes, 3 speeds) was performed to determine the influence of shortening amplitude and shortening rate on both the transient and steady-state aspects of FD, in both wild-type and jump muscle expressing EMB. EMB-expressing TDTs showed nearly twice the amount of FD across all active shortenings with slower rates of force redevelopment when compared to TDTs with wild-type myosin. These results strongly implicate myosin kinetics at the core of the underlying FD mechanism.

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Biochemical Diversity of Human Skeletal Muscle

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INTRODUCTION

The molecular components largely responsible for muscle attributes such as passive tension development (titin and collagen), active tension development (myosin heavy chain [MHC]), and mechanosensitive signaling (titin) are well studied in animals. We performed a comprehensive analysis of these components in human muscle to search for common themes and trends in the muscular organization of the human body.

METHODS

599 biopsies from 6 donors were obtained early postmortem. Three assays were performed on each biopsy - titin molecular weight determination (n=586), hydroxyproline content (a surrogate for collagen content) (n=599), and MHC isoform distribution (n=599).

Muscles were analyzed individually and in functional groups. Analysis of variance (ANOVA) was used to compare individual muscles and discriminant function analysis (DFA) was used to determine which dependent variables were the best predictors of muscle group.

RESULTS

DFA demonstrated that titin MW was the strongest predictive factor of anatomic region and muscle functional group.

On average, human muscles were very ''slow'' (i.e., had more slow- myosin than muscles of lower mammals). Average % MHC-1 of muscles in this study was 65% (compare to 6% for mouse, 8% for rat, and 19% for rabbit). Overall, larger titins were associated with faster muscles.

DISCUSSION:

The finding that titin was the strongest discriminating factor for DFA with anatomic region and muscle functional group as grouping variables is unexpected; titin is currently primarily considered to be a signal transduction cascade activator or determinant of passive tension. The idea that titin MW is a good predictor of muscle anatomic localization is previously unexplored and warrants further study.

The titin-% MHC-1 relationship we observed is opposite to that previously reported in rabbits, where large titins were associated with slower muscles. This may represent a different design paradigm in human vs rabbit muscular organization.

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Extracellular Adaptations to Altered Force Transmission in Muscle Cells

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Interaction between active force generating components and passive extracellular matrix (ECM) force transmitting components provides skeletal muscle with its characteristic biomechanical properties, enabling it to produce work and resist extension. However, the mechanisms behind this ''communication'' and the cellular structures that enable it are poorly understood. Desmin is an intermediate filament protein integral to the muscle fiber cytoskeleton. It is thought to be involved in stabilizing the contractile apparatus and transmitting force between the cytoskeleton and the ECM. In this study, we investigated the effects of desmin deletion (des-/-) on the composition and mechanical properties of the cellular and extracellular components of muscle.

We found that, while des-/- and wt muscles have identical fiber and bundle (fibers+ECM) material properties at birth, these properties diverge becoming disparate with age. Specifically, des-/- fibers become more compliant compared to wt fibers (tangent modulus: 42.8 ± 6 kPa/um and 66.8 ± 7 kPa/um respectively), while des-/- bundles become progressively stiffer compared to wt (tangent modulus: 193.3 ± 19 kPa/um and 129 ± 13 kPa/um respectively). This result suggests that des-/- muscles are chronically altering their ECM. Evidence supporting this hypothesis was provided by significantly increased collagen protein content (des-/-: 8.0 ± 1 ug/mg, wt: 3.4 ± 1 ug/mg), ECM area fraction (des-/-: $15.1 \pm 1\%$, wt: $10.5 \pm 1\%$) and fibrosis-related gene expression patterns in des-/- muscle compared with wt. Additionally, significantly increased numbers of centrally nucleated fibers (des-/-: $5.6 \pm 0.5\%$, wt: $0.7 \pm 0.2\%$) and inflammatory gene expression patterns in des-/- muscle suggest that an increased susceptibility to injury in des-/- fibers may underlie the measured fibrotic response. Thus, in this study, we identify a possible causal relationship between increased cellular compliance, due to reduction in force transmission and stabilization by desmin, and the proliferative response of the extracellular matrix cellular constituents.

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A Comparison of Single Myofibrils and Single Muscle Fibers from Rabbit Psoas in Terms of MgATP Binding and Cross-Bridge Detachment Steps Studied with Sinusoidal Length Perturbations

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Single myofibrils of 40-70 μm in length and 2-4 μm in diameter were isolated from rabbit psoas muscle bundles, and cross-bridge kinetics were studied by